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TITLE: Mouse and Human Models for Investigating Influences of Tau on Progression of Alzheimer's Disease Following Traumatic Neuronal Injury

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

We have completed the design, fabrication, and validation of a new biomedical device to impose moderate mechanical loads on cultured stem-cell derived neuronal and glial cells. Using this device, we assessed morphological changes, beta amyloid production, and tau phosphorylation (i.e., multiple Alzheimer's-associated outcomes) following rapid stretch of iPSC-derived neurons. Results suggest that neurites oriented in the direction of substrate stretch were non-elastically deformed, the cytoskeleton was reorganized, amyloid precursor protein transport was perturbed, amyloid production was increased, and tau phosphorylation unchanged following a single bout of mechanical loading. Multiple bouts of mechanical loading amplified amyloid and tau phenotypes, suggesting a dependence of these Alzheimer's associated outcomes to injury dose or severity.

15. SUBJECT TERMS

Traumatic brain injury, neuron, biomechanics, tau, amyloid, stretch, microfluidic device, Alzheimer's Disease, stem cell, iPSC

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Table of Contents

The text of the report must include all sections addressed in the table of contents to include the following. **DO** include the bolded section headings, but **DO NOT** include the *italicized* descriptions of section contents in your submitted reports.

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

   This proposal aims to examine mechanisms underlying the progression of Alzheimer's following TBI. We hypothesize that persistent changes in the phosphorylation state of tau, a protein that stabilizes microtubules in the neuronal cytoskeleton when unphosphorylated, is a key node in regulating the development of AD outcomes after TBI, both during the primary mechanical injury and also the secondary inflammatory response. We propose the integration of a preclinical mouse model of TBI, bioengineered devices to mechanically damage human induced pluripotent stem cell (iPSC)-derived neurons, genetic and pharmacological manipulation of tau and its kinases in iPSC-derived neurons, and quantitative biochemical, cell biological, imaging, and functional assessment of outcomes. This multi-disciplinary strategy is designed to examine the interlinked contributions of tau, inflammatory cytokines, and cytoskeletal instability on the development of TBI-induced AD. Our specific aims are: Aim 1: To test the effect of modulating tau expression and phosphorylation on AD-associated phenotype development in vitro on human iPSC-derived neurons submitted to mechanical-induced injury (primary injury) with and without treatment with specific inflammatory cytokines (secondary injury).

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

   Traumatic brain injury, neuron, biomechanics, tau, amyloid, stretch, microfluidic device, Alzheimer's Disease, stem cell, iPSC, cytokine, inflammation

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.*

   - **What were the major goals of the project?**

     Per the SOW, our Aims/Milestones were (Year and quarter in parentheses):

     M0.1: IRB Exemption for human iPSC Studies (Y1,Q1)
     M0.2: HRPO Review (Y1,Q1)
     M1.1. Generation of tau mutant cells (Y1, Q2-Y1, Q4)
     M1.2. Characterization of AD-associated outcomes in unstretched and rapidly stretched neurons from groups expressing varying levels of tau, at each time point. (Y1, Q4-Y2, Q4)
     M1.3. Characterization of AD-associated outcomes in unstretched and rapidly stretched neurons from groups with varied tau phosphorylation or kinase activation, at each time point. (Y2, Q4 – Y3, Q4)
     M1.4. Statistical comparison of outcomes from mechanically stretched groups. (Y3, Q4 – Y3, Q4)
     M1.5. Characterization of AD-associated outcomes in cytokine-treated neurons from groups expressing varying levels of tau, at each time point. (Y1, Q4-Y2, Q4)
     M1.6. Characterization of AD-associated outcomes in cytokine-treated neuronsfrom groups expressing varying tau phosphorylation or kinase activation, at each time point. (Y2, Q4 – Y3, Q4)
     M1.7. Statistical comparison of outcomes in cytokine treated groups. (Y3, Q4 – Y3, Q4)

   - **What was accomplished under these goals?**

     - *1) major activities, 2) specific objectives, 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative)*

     - *We have completed milestone 0.1 and 0.2. UCSD IRB and HRPO granted approval of our resubmitted study.*
     - *In the previous annual report, we described the completed design and characterization of our cellular loading device. We also described morphological phenotypes (axonal waviness) associated with traumatic mechanical loading and preliminary data suggesting an increase in beta-amyloid levels 24 hours after injury.*
     - *In this period, we achieved several research goals.*

1. Quantified axonal strains and the relationship between axonal waviness and axonal orientation.

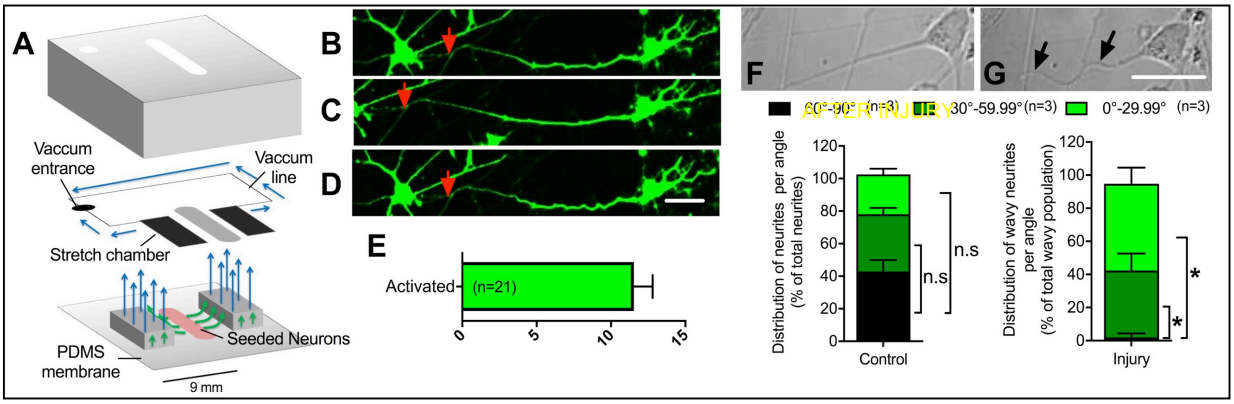

Fig 1. (*A*) Schematic depicting novel biomedical device used in our studies. Briefly, after vacuum line is activated, suction displaces the walls flanking the cell culture chamber (in red), which pulls bilaterally the PDMS membrane and attached cells located between them. (B-D) Control hiPSC-derived neurons transfected with GFP were imaged before stretch (B), while holding stretch (C), and immediately after releasing tension (D). (E) % of increase of neurite length measured while holding stretch. (Mean + SEM; n=21 neurites). (F) Brightfield images depict neurite's undulations appearance immediately after stretch (G; arrows). (Bottom graphs) The orientation of neurites in cultured neurons in our devices is random (left). Neurites oriented in the direction of stretch are most severely affected (Right). (Mean +/- SEM). Scale bar = 50 mm

2. Observed reversible redistribution of tubulin localization after injury and confirmed that injury was sublethal, based on cell toxicity assays (CellTiter Glo, data not shown) and live imaging of Ca++ binding dye.

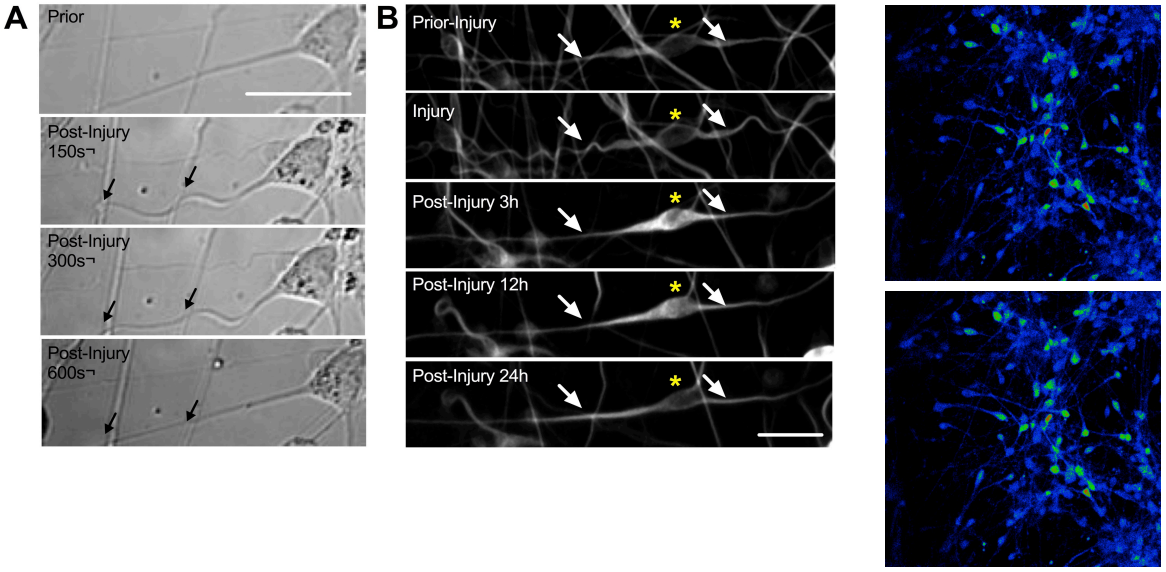

Fig 2. Brightfield images illustrate fast recovery of injury-induced neurite waves within 10 minutes post-injury (A). Sir-Tubulin staining in hiPSC derived neurons, demonstrate tubulin translocation from neurites to neuron's cell body within 3 hr post-injury and reversal 24 hr post-injury (B). Scale bars = 50 mm. (C) Calcium was labeled with fluo-4, and displayed no change in pattern before and after injury, suggesting that membrane integrity remained intact with strain.

3. Observed change in geometry and spacing of APP localization 24 hours after injury, indicative of altered fast axonal transport. Neurofilament patterns were unchanged (data not shown).

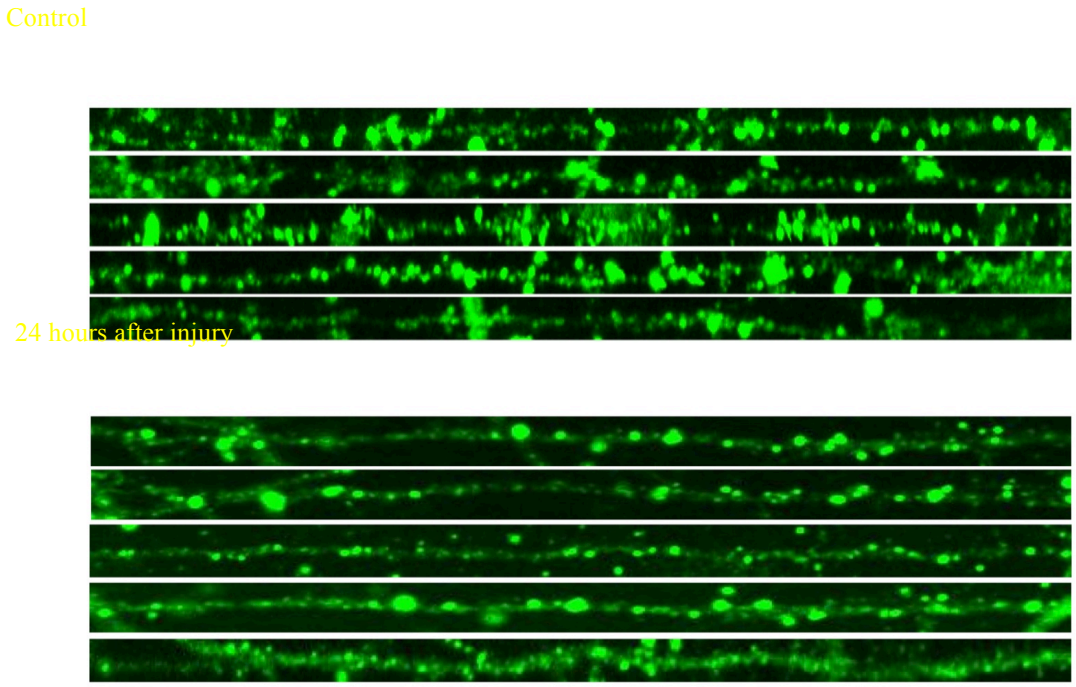

Fig 3. APP Immunostaining in axons from hiPSC derived neurons, fixed 24 hrs after no injury (top) or post-injury (bottom). Note differences in spacing and geometry of puncta.

4. Observed differences in amyloid and phosphor-tau response following a single injury or repeated (3x) daily injuries. After a single injury, amyloid beta 1-42 (Abeta42) and the ratio of Abeta42 to Abeta 40 increased after 24 hours, before returning to basal levels at 48 hours and later. No increase in P-tau was observed. In contrast, after repeated injury, Abeta increases were amplified, and P-tau and P-tau/tau increases were observed.

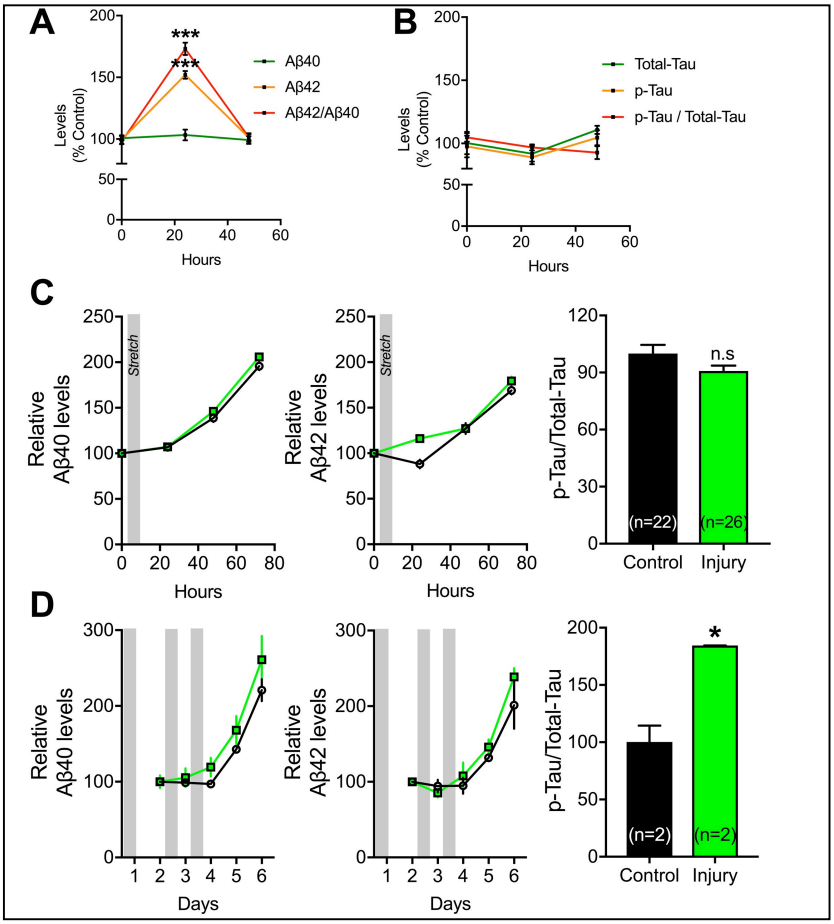

Fig 4. Levels of secreted Ab (A) and intracellular total and P-tau at Thre231 (B) were measured using MSD plates at 24 and 48 hr after injury (mean+/-SEM; n = 25, 4 neuronal differentiations). (C-D) Levels of secreted Ab40 (left graph), Ab42 (middle graph) at time =0, 24, 48, 72 hr, and intracellular total tau and P-tau (right graphs) at 72h were measured using MSD plates after 1x bout of injury (C; one grey vertical line), or repetitive injuries (D; 3 grey vertical lines).

5. Our findings have enabled us to formulate a working hypothesis, that β-amyloid may be a sensitive indicator of risk for AD-associated phenotypes following TBI.

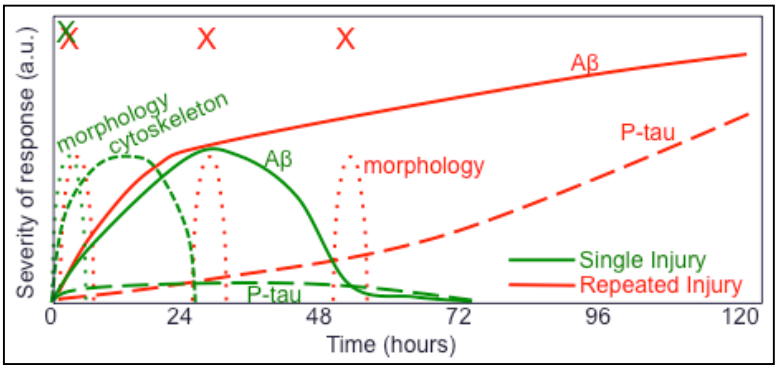

Fig 5. Working hypothesis: β-amyloid may be a sensitive indicator of risk for AD-associated phenotypes following TBI. This schematic summarizes morphological, cytoskeletal, amyloid, and P-tau response for single (green) and multiple (red) injuries.

- *4) other achievements. Nothing to report.*

- **What opportunities for training and professional development has the project provided?**
  - *One postdoctoral fellow and one Master's student participated in the project in the past reporting period. They have received technical training related to engineering and stem cell biological aspects of the proposed research as well as opportunities to present their findings at lab meetings and departmental seminars. In addition, the PI and Co-PIs have mentored these individuals in activities associated with literature review, data analysis, and writing.*
- **How were the results disseminated to communities of interest?**
  - *Preliminary findings have been disseminated at lab meetings and departmental seminars.*
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - *We will continue to execute our research plan as proposed.*

6. **IMPACT:** *Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

- **What was the impact on the development of the principal discipline(s) of the project?**
  - *Our progress thus far impacts the intersection of biomedical device design and stem cell biology. Our development of a new mechanical loading device offers the traumatic neuronal injury field a new tool to reliably impose moderate injury to cultured stem-cell derived neuronal and glia cells, and examine biological outcomes with high sensitivity and at high resolution.*
  - *Our observation that a single bout of injury results in an amyloid phenotype, and multiple bouts of injury result in both amyloid and tau phenotypes suggests a dose dependence of Ad-associated outcomes with the frequency and/or severity of injury.*
- **What was the impact on other disciplines?**
  - *Nothing to report*
- **What was the impact on technology transfer?**
  - *Nothing to report.*
- **What was the impact on society beyond science and technology?**
  - *Nothing to report.*

7. **CHANGES/PROBLEMS:** *The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

   - **Changes in approach and reasons for change**
     - *None to date.*
   - **Actual or anticipated problems or delays and actions or plans to resolve them**
     - *None to date.*
   - **Changes that had a significant impact on expenditures**
     - *None.*
   - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**
     - *None.*
   - **Significant changes in use or care of human subjects**
   - **Significant changes in use or care of vertebrate animals.**
   - **Significant changes in use of biohazards and/or select agents**

8. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

   - **Publications, conference papers, and presentations**
     Report only the major publication(s) resulting from the work under this award.
     - **Journal publications.** *None to date.*
     - **Books or other non-periodical, one-time publications.** *None to date.*
     - **Other publications, conference papers, and presentations.**

       We presented pilot data at the Society for Neuroscience Annual Meeting in San Diego, CA: Balcer, A., dos Santos Chaves, R., Gutierrez, E., Groisman, A., Goldstein, L., Almenar-Queralt, A., Shah, S.B. Ex-vivo modeling of Alzheimer's Disease outcomes following traumatic mechanical injury of human iPSC-derived neurons. 2016 Society for Neuroscience Annual Meeting, San Diego, CA. November 15, 2016.
   - **Website(s) or other Internet site(s)**
     None to date.
   - **Technologies or techniques**
     Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.
   - **Inventions, patent applications, and/or licenses**
     None to date.
   - **Other Products**
     None to date.

   - **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
   - **What individuals have worked on the project?**

- *Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."*

Name: Sameer Shah (12%)
Project Role: PI
Researcher Identifier: sbshah@ucsd.edu
Nearest person month worked: 2
Contribution to Project: Completion of regulatory documents, communication with Dr. Hein/Grate and HRPO, project planning to account for delays in HRPO approval, data analysis.

Name: Larry Goldstein (5%)
Project Role: Co-PI
Researcher Identifier: lgoldstein@ucsd.edu
Nearest person month worked: 0.25
Contribution to Project: Completion of regulatory documents, project planning, interpretation of results.

Name: Angels Almenar-Queralt (8%)
Project Role: Co-PI
Researcher Identifier: aalmenar@ucsd.edu
Nearest person month worked: 1
Contribution to Project: Completion of regulatory documents, project planning, data analysis, interpretation of results.
.

Name: Brian Head (3%)
Project Role: Co-I
Researcher Identifier: bhead@ucsd.edu
Nearest person month worked: 0.25
Contribution to Project: Pilot experimentation, additional literature review.

Name: Rodrigo Chaves (100%)
Project Role: Postdoc
Researcher Identifier: rchaves@ucsd.edu
Nearest person month worked: 12
Contribution to Project: Pilot experimentation, Experiments described above, data anlaysis, interpretation of results, dissemination of results.

Name: Andrew Holder (50%)
Project Role: Master's student
Researcher Identifier: andrewholder51@gmail.com
Nearest person month worked: 3
Contribution to Project: Pilot experimentation, Experiments described above, data anlaysis, interpretation of results, dissemination of results.

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

  Nothing to report

  - **What other organizations were involved as partners?**

Nothing to Report

- **Describe partner organizations - academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) - that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed. Provide the following information for each partnership:**

  Nothing to Report

9. **SPECIAL REPORTING REQUIREMENTS**

   - **COLLABORATIVE AWARDS:** *For collaborative awards, independent reports are required from **BOTH** the Initiating PI and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to https://ers.amedd.army.mil for each unique award.*

   - **QUAD CHARTS:** *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

     Nothing to Report

10. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc. Reminder: Pages shall be consecutively numbered throughout the report. **DO NOT RENUMBER PAGES IN THE APPENDICES.***

    Nothing to Report